Cells as Machines: Towards Deciphering Biochemical Programs in the Cell (keynote talk)

François Fages

Cells as Machines: towards deciphering biochemical programs in the cell

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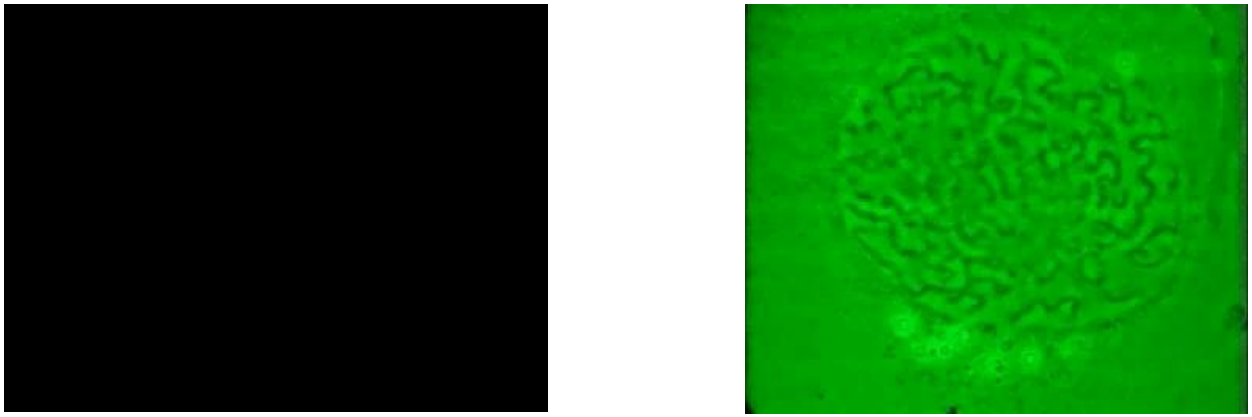
To tackle the complexity of biochemical reaction systems, investigate:

- Programming theory concepts
- Formal methods of circuit and program verification
- Artificial Intelligence techniques

Implemented in BIOCHAM v3.5 (Biochemical Abstract Machine)

Systems Biology Challenge

Gain system-level understanding of multi-scale biological processes in terms of their elementary interactions at the molecular level. [Kitano 1999]



Cell mitosis videos [Lodish et al. 03]

Follow-up of Human Genome Project (90s)

→ Beyond genomic data: protein-protein interaction databases, RNAs, …

→ Model repositories of cell processes : e.g. **`biomodels.net`** 1000 models

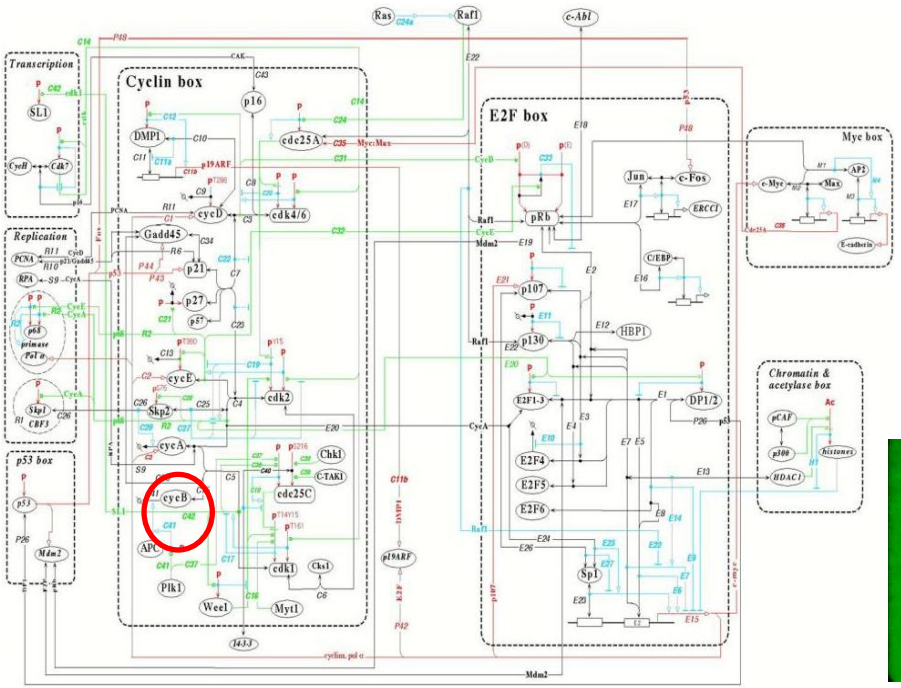
→ Systems Biology Markup Language (SBML): model exchange format

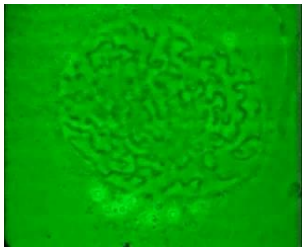
→ Modeling environments (Cell designer, Cytoscape, Copasi, BIOCHAM,…)

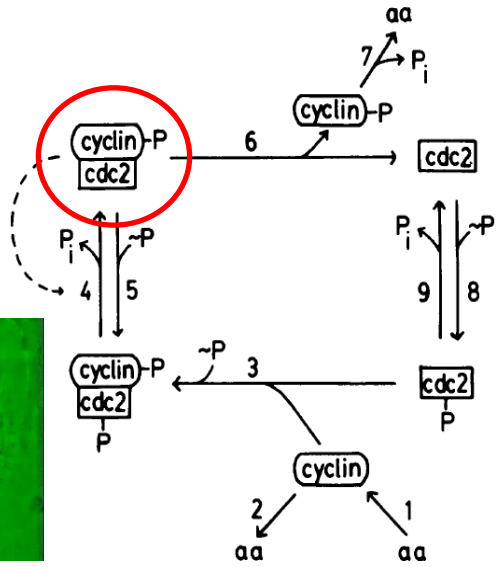
→ Simulation of a whole-cell *mycoplasma genitalium* [Karr Covert et al 12]

Biochemical reaction models

1) Models for representing knowledge : the more detailed the better
2) Models for answering questions : the more abstract the better







Mammalian cell cycle control interaction map [Kohn 1999]

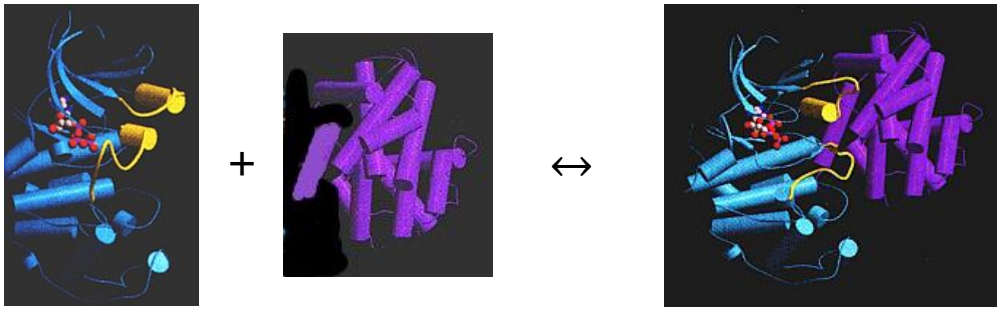
Simplified yeast cell cycle model [Tyson 1991]

Biochemical reactions

- Binding, complexation: $A + B \rightarrow C$

  $cdk1 + cycB \rightarrow cdk1cycB$

- Unbinding, decomplexation: $A \rightarrow B + C$



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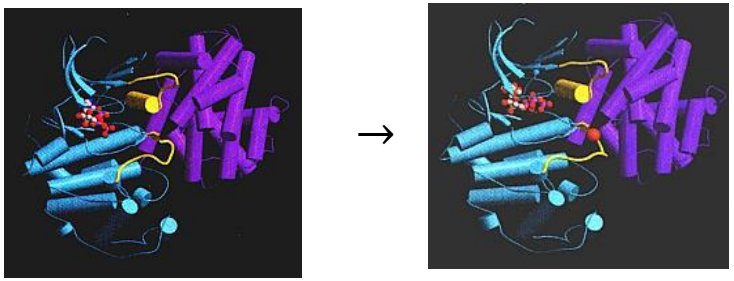

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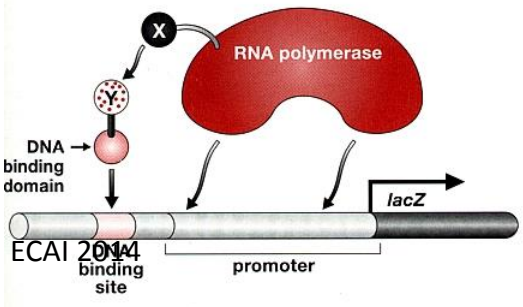

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Biochemical reaction rates

Time matters

- Binding, complexation:

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  $A + B \xrightarrow{k.A.B} C$

- Unbinding, decomplexation:

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- Transformation, phosphorylation, transport:

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Biochemical reaction rates

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Mass action law kinetics

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Michaelis Menten kinetics

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Hill sigmoid: switch like behavior

- Gene expression, synthesis: $A \xrightarrow{v.A^n/(k+A^n)} A + B$

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Semantics of Reactions $A+B \xrightarrow{f(A,B)} C$

Boolean Semantics: presence-absence of molecules $A \wedge B \rightarrow C \wedge A/\neg A \wedge B/\neg B$

Asynchronous Transition System

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Petri Net Semantics: numbers of molecules $A , B \rightarrow C{+}{+}, A{-}{-}, B{-}{-}$

- Multiset rewriting
- CHAM [Berry Boudol 90] [Banatre Le Metayer 86]

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Stochastic Semantics: reaction probabilities, time of next reaction in a given state S_i

- Continuous Time Markov Chain (CTMC) $A , B \xrightarrow{p(S_i),\, t(Si)} C{+}{+}, A{-}{-}, B{-}{-}$

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Logical time

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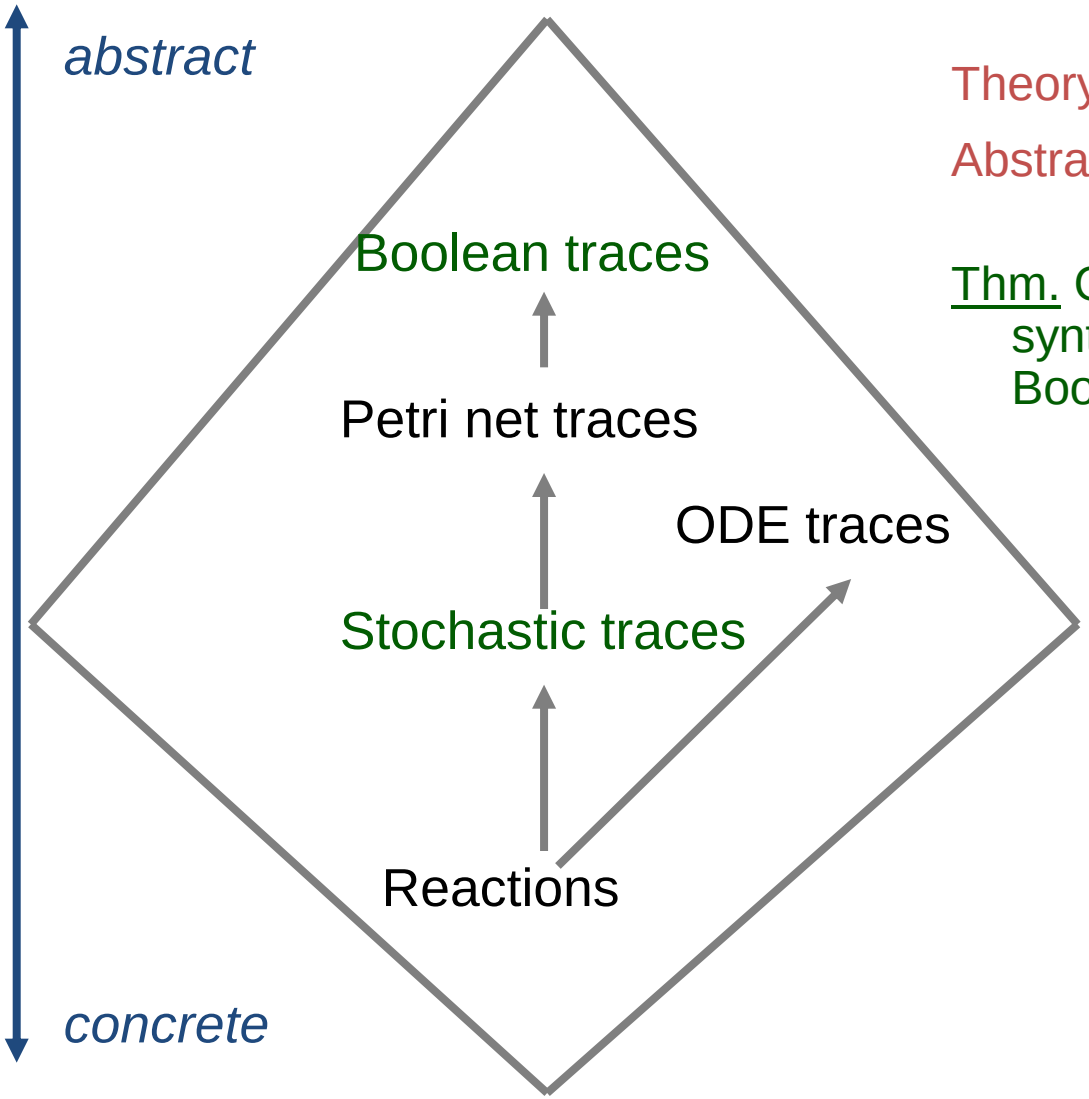
Continuous Semantics: concentrations, continuous evolution

Ordinary Differential Equations (ODE) $\dot{A}_i = \sum_{r=1}^{n} \mathrm{f}_r \times \delta_r(Ai)$

Abstraction Relationships



Theory of abstract Interpretation

Abstractions as Galois connections

[Cousot Cousot POPL'77]

Thm. Galois connections between the syntactical, stochastic, Petri Net and Boolean semantics

[FF Soliman CMSB'06,TCS'08]

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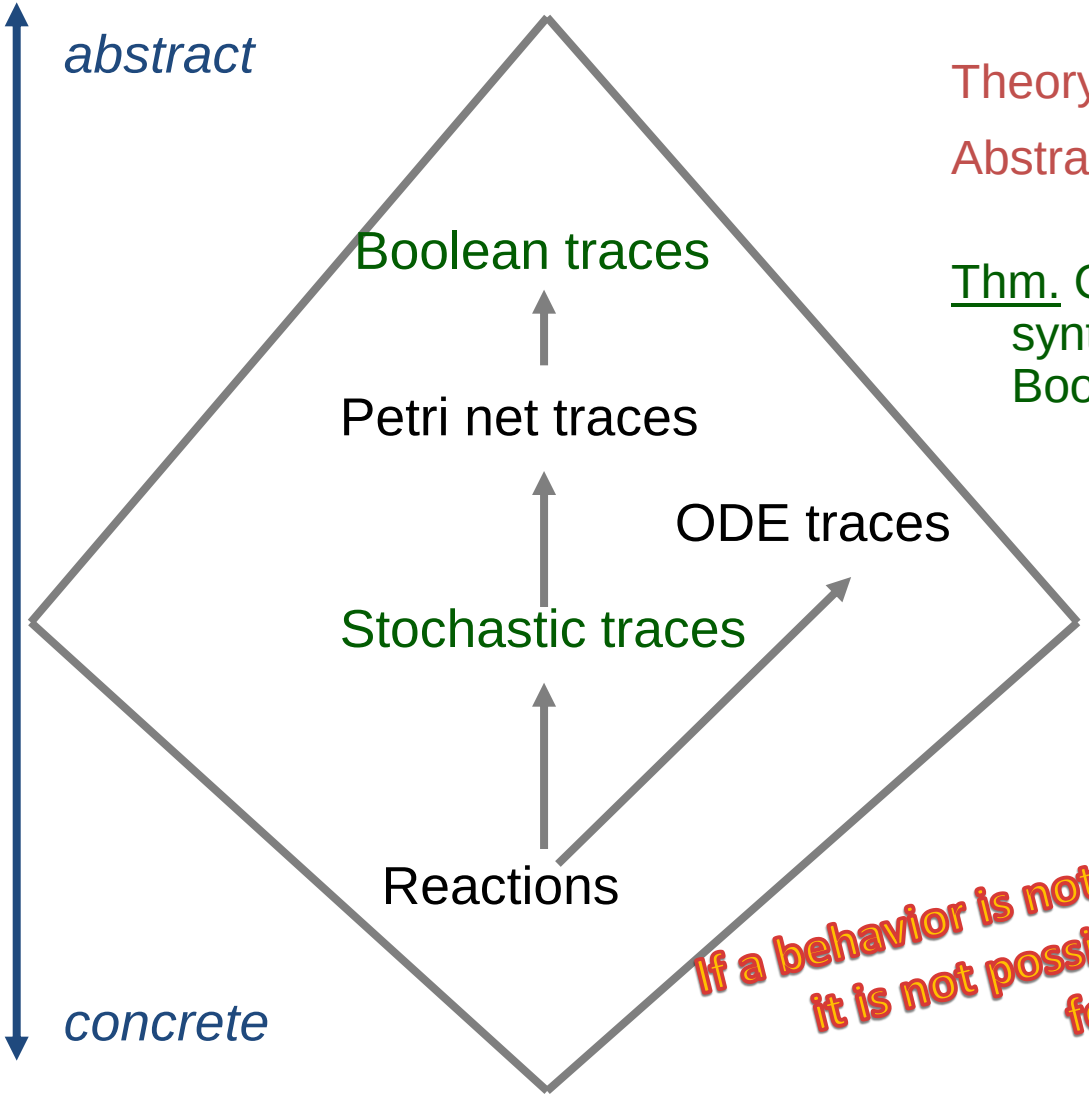

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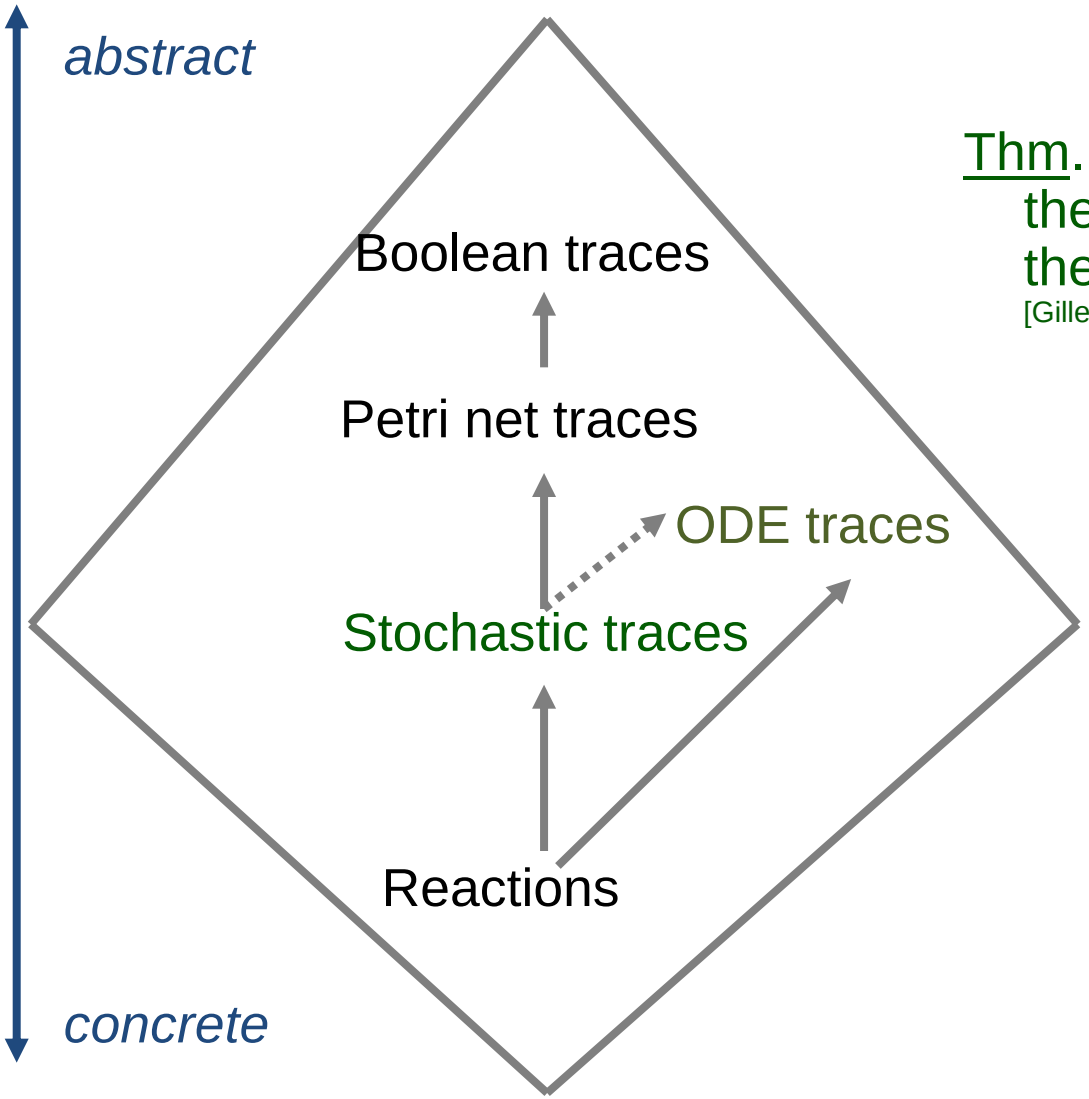
If a behavior is not possible in the Boolean semantics it is not possible in the stochastic semantics for any reaction rates

Abstraction Relationships



Thm. Under large number conditions the ODE semantics approximates the mean stochastic behavior
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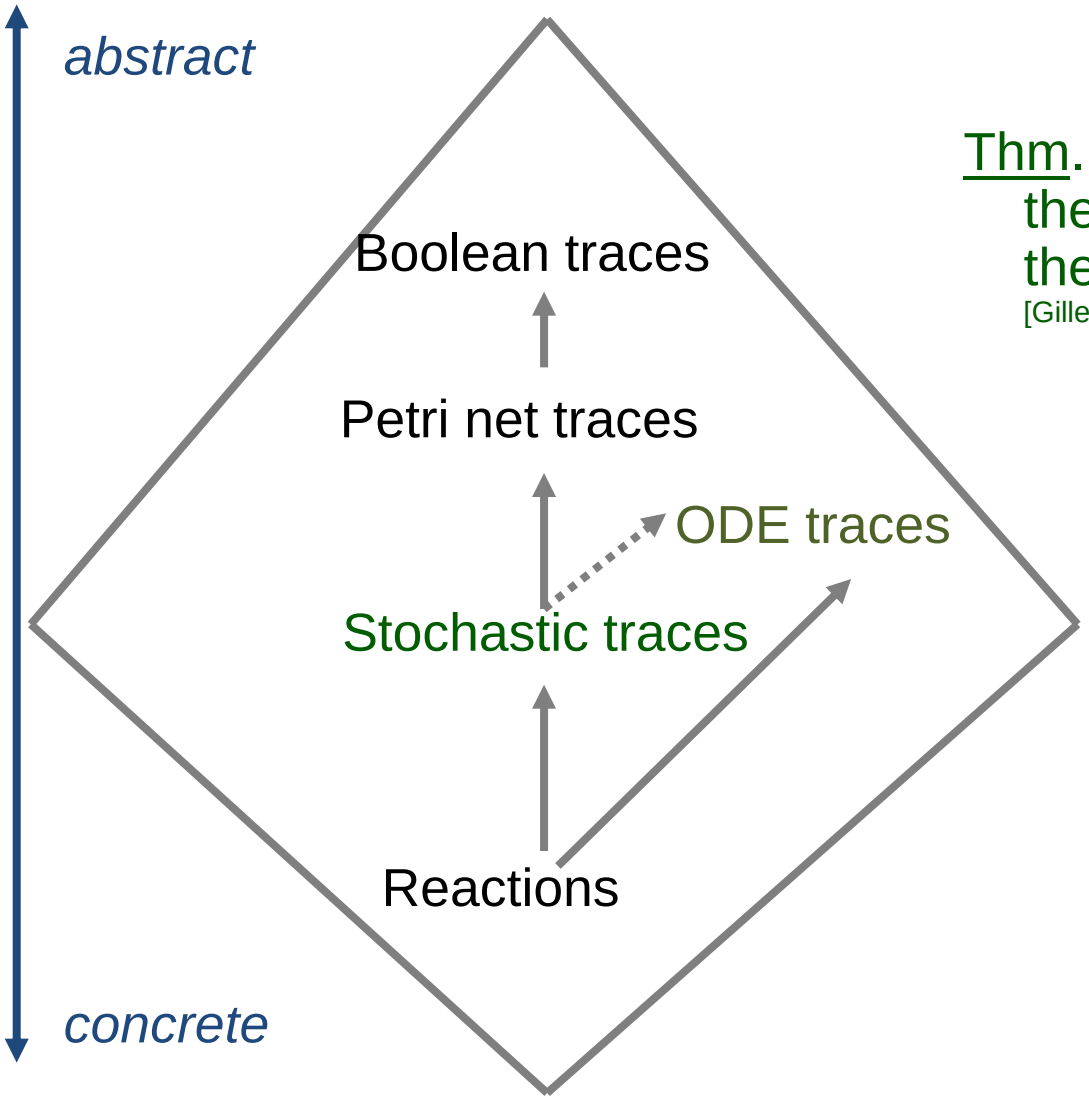

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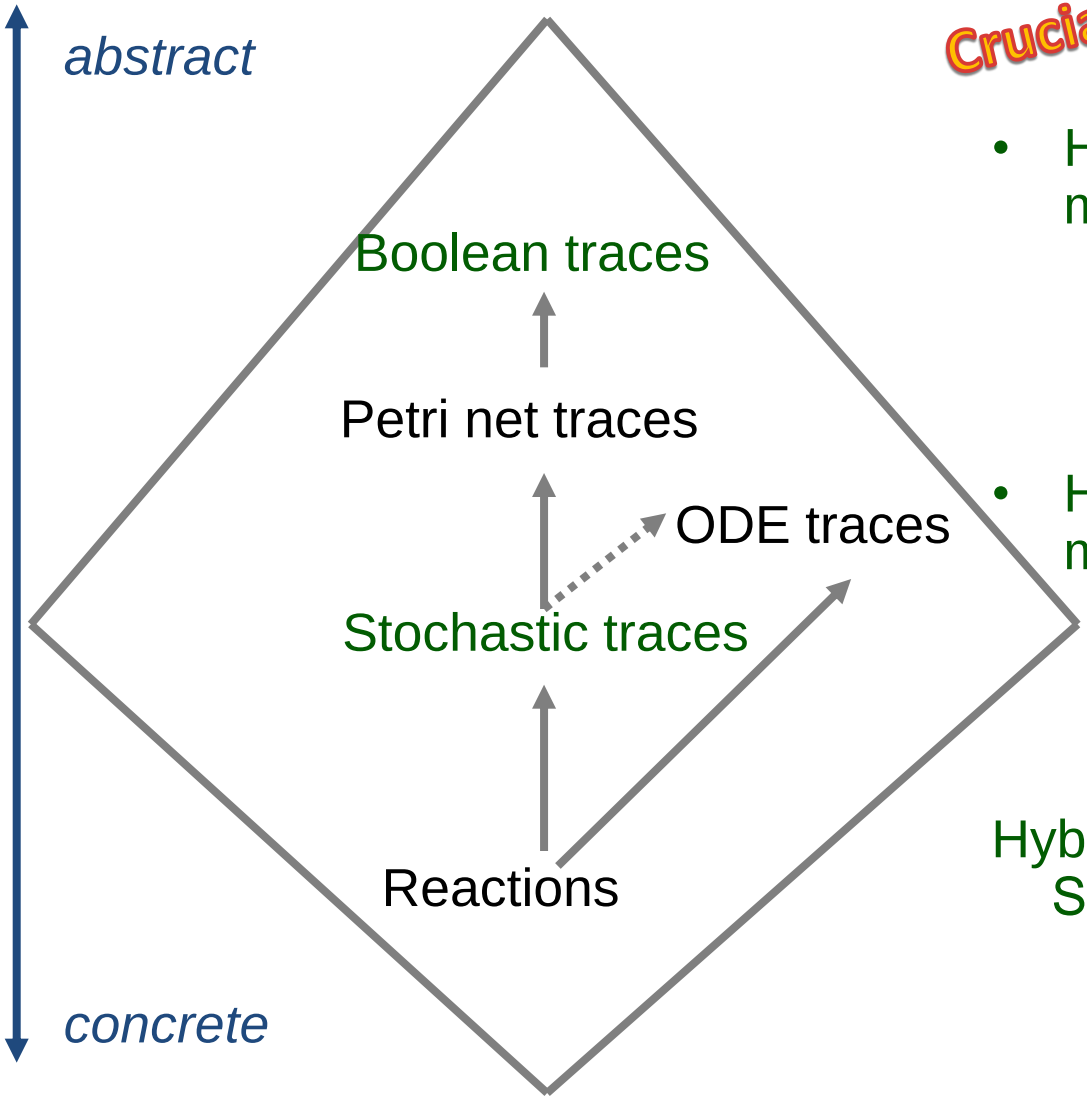
Hot topic:
higher order moments
ODE for mean, variance,…

Hybrid Models



Crucial for model reduction and multi-scale models

- Hybrid Boolean-continuous models (hybrid automata)

  Boolean gene expression + continuous protein activation

- Hybrid stochastic-continuous models (CTMC+ODE)

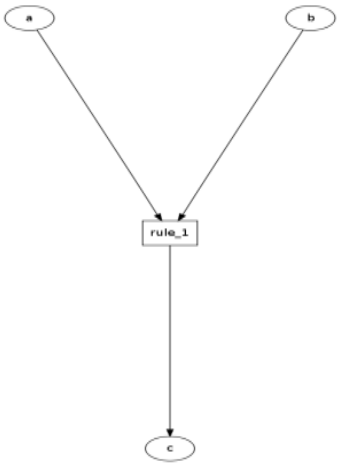
  Stochastic gene expression + continuous protein activation

Hybrid simulators for free with SBML's reactions+events

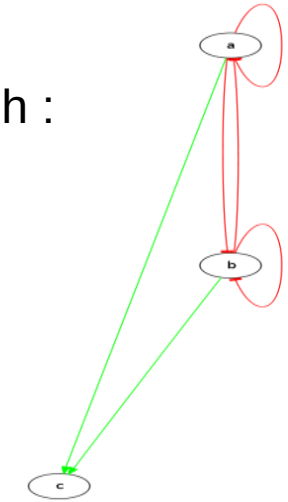
[Chiang FF Huang Soliman 13 cmsb]

Before going to simulations: structural analyses

- Reaction hypergraph :
  - $A + B \rightarrow C$



Influence graph :



  - Large independence of the influence graph from the kinetics [FF Soliman 08 fmsb]

- Existence of positive circuits in the influence graph

  → necessary condition for multi-stationarity (cell differentiation, homeostasis) in Boolean and ODE semantics [Soliman 13 bmb, Soule 03, Thomas 82]

- Existence of negative circuits in the influence graph

  → necessary condition for oscillations in ODE semantics [Snoussi 93, Thomas 82]

Structural analysis: reaction graph reduction

- Subgraph epimorphisms (SEPI) [Gay FF Martinez Soliman 13 dam]
  - Structural notion of model reduction by deletion & merge of species & reactions in reaction hypergraphs

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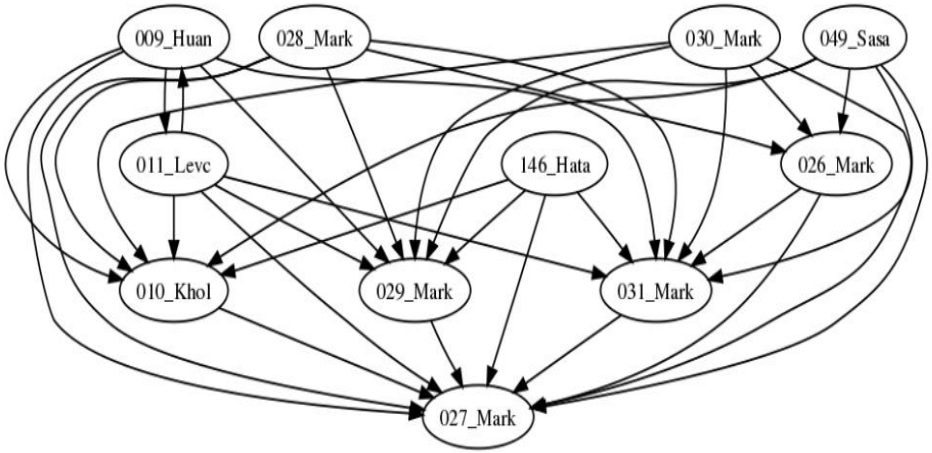
  - Reconstructed hierarchy of MAPK models in biomodels.net [Gay Soliman FF 10 bi]



Possibility to query model repositories by the structure of reaction networks

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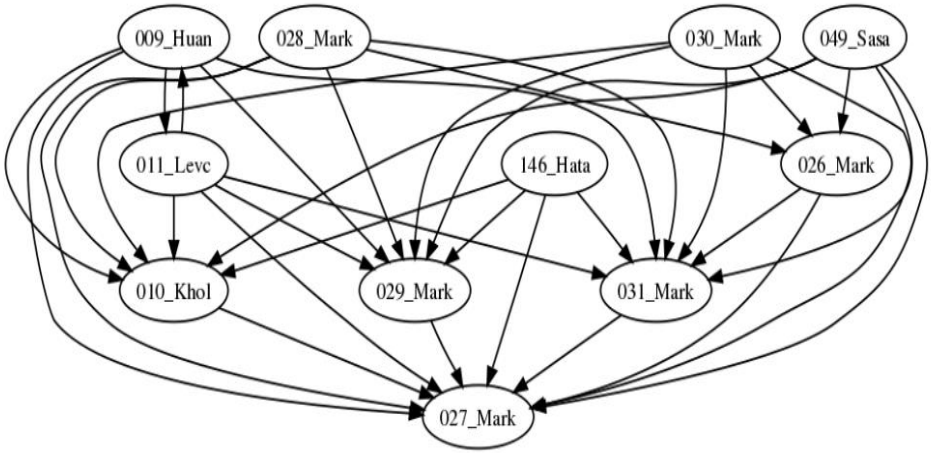

Possibility to query model repositories by the structure of reaction networks

- The SEPI existence problem is NP-complete

Quite efficient computation of SEPI by CLP(FD) /SAT solvers [Gay FF Santini Soliman 13 wcb]

Structural analysis: Petri net invariants

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The existence of a minimal siphon containing a given place is NP-complete

However, very efficient enumeration of all minimal siphons in all biomodels.net by CLP(FD) and/or SAT solvers [Nabli Martinez FF Soliman 12 CP]

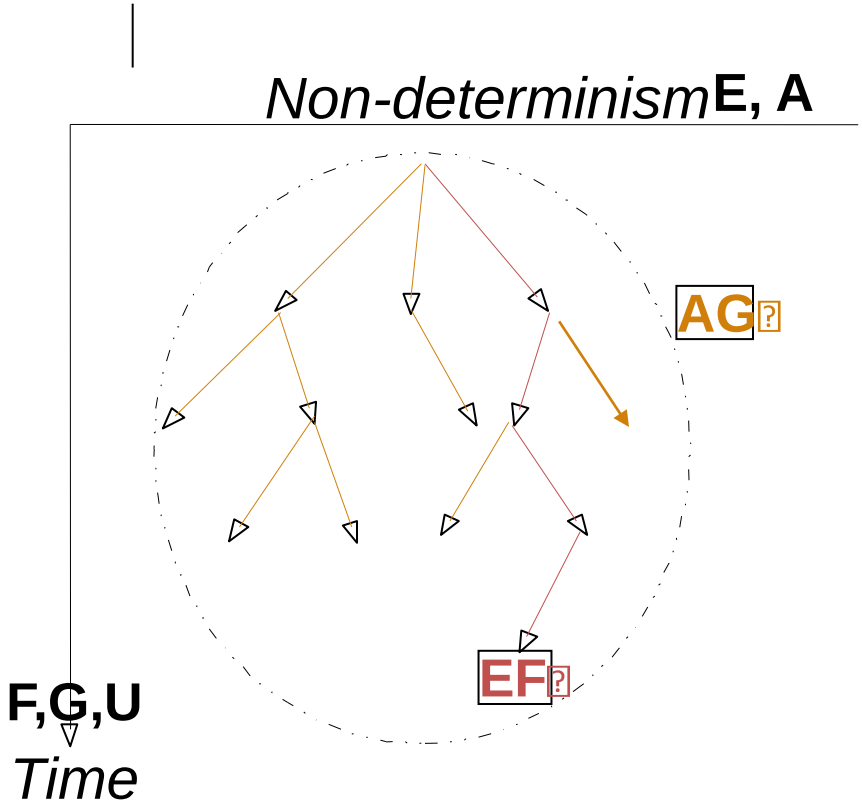
Structural analysis of dynamical behaviors

- Temporal logic FO patterns
  - Stability **G**φ
  - Reachability **F**φ , thresholds **F**([A]>0.1),
  - Peaks of concentration **F**([A]<V ∧ **X**([A]=V ∧ **X**([A]<V))
  - Amplitude
  - Periods and phases as distance between peaks
  - …
- More flexible than curve fitting, can deal with imprecise data
- Constraints for model validation/synthesis w.r.t. experimental observations
- Constraints for parameter search
  - Good fit → model-based predictions
  - No good fit → revisit structure → model-based contribution to biology

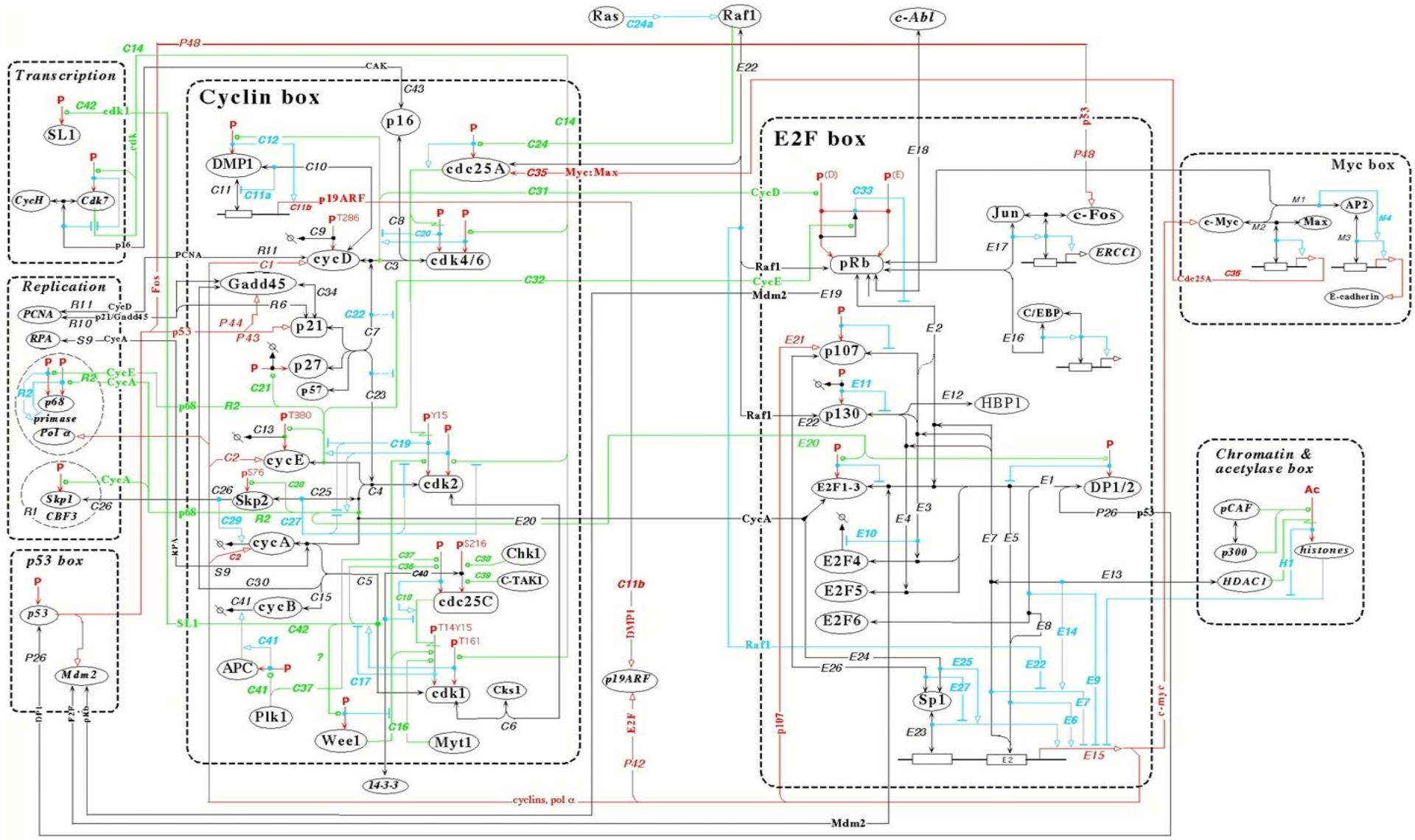
Boolean Semantics Queries

Computation Tree Logic CTL [Emerson Clarke 80]

Non-det. Time	**E** exists	**A** always
X next time	**EX**(φ)	**AX**(φ)
F finally	**EF**(φ) ¬ ***AG***(¬ φ)	**AF**(φ) *liveness*
G globally	**EG**(φ) ¬ ***AF***(¬ φ)	**AG**(φ) *safety*
U until	**E** (φ1 **U** φ2)	**A** (φ1 **U** φ2)



Mammalian Cell Cycle Control Map [Kohn 99]



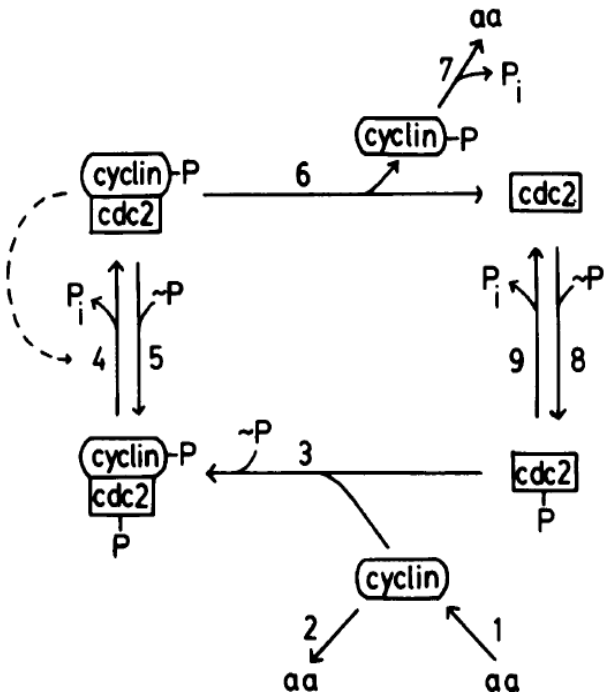
Kohn's Map Boolean Model-Checking

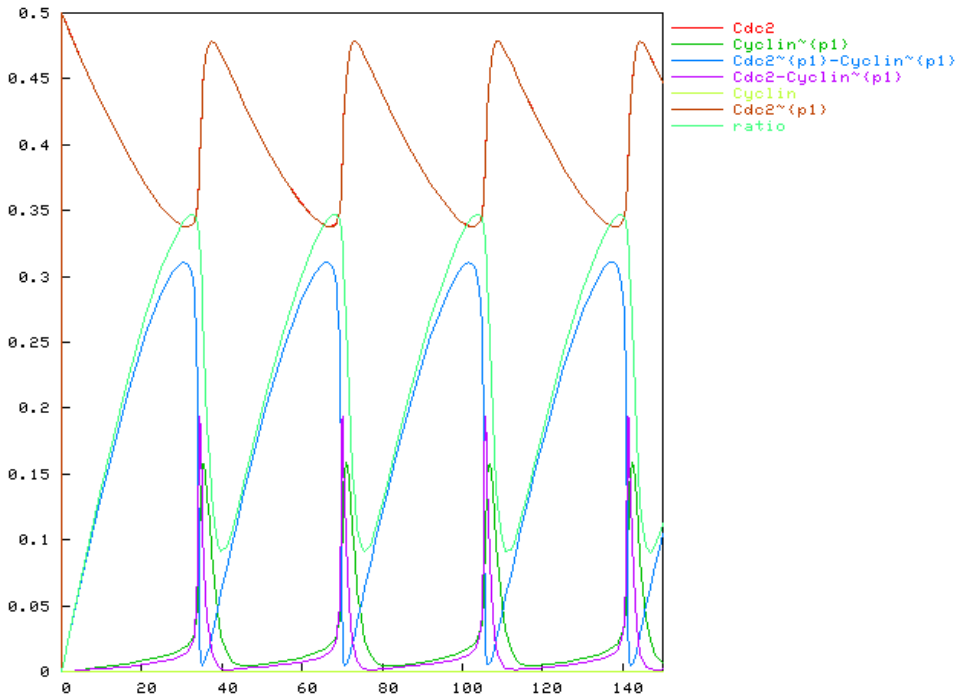
800 reactions, 165 proteins and genes, 500 variables, 2^{500} states.

Biocham NuSMV symbolic model-checker time in seconds [Chabrier et al. TCS 04]

Initial state G2	Query:	Time in sec.
	compiling	29
Reachability G1	**EF** CycE	2
Reachability G1	**EF** CycD	1.9
Reachability G1	**EF** PCNA-CycD	1.7
Checkpoint for mitosis complex	¬ **EF (**¬ Cdc25~{Nterm} **U** Cdk1~{Thr161}-CycB**)**	2.2
Oscillations CycA	**EG ((EF** ¬ CycA**) & (EF** CycA**))**	31.8
Oscillations CycB	**EG ((EF** ¬ CycB**) & (EF** CycB**))**	6

Dynamical Model of Cell Cycle Control [Tyson 91]





6 molecular species

8 reactions

8 kinetic parameters

ODE semantics

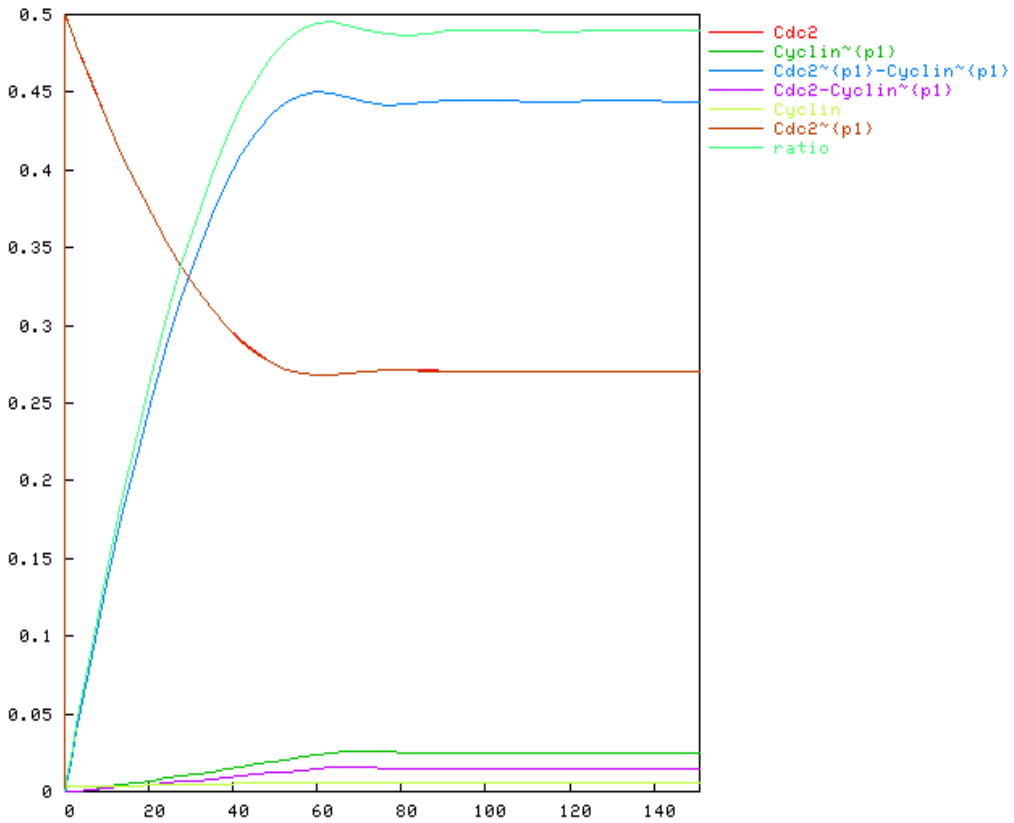
Parameter Search from LTL(R) Properties

biocham: search_parameter([k3,k4],[(0,200),(0,200)],20,
oscil(Cdc2-Cyclin~{p1},3),150).

First values found :

parameter(k3,10).

parameter(k4,70).



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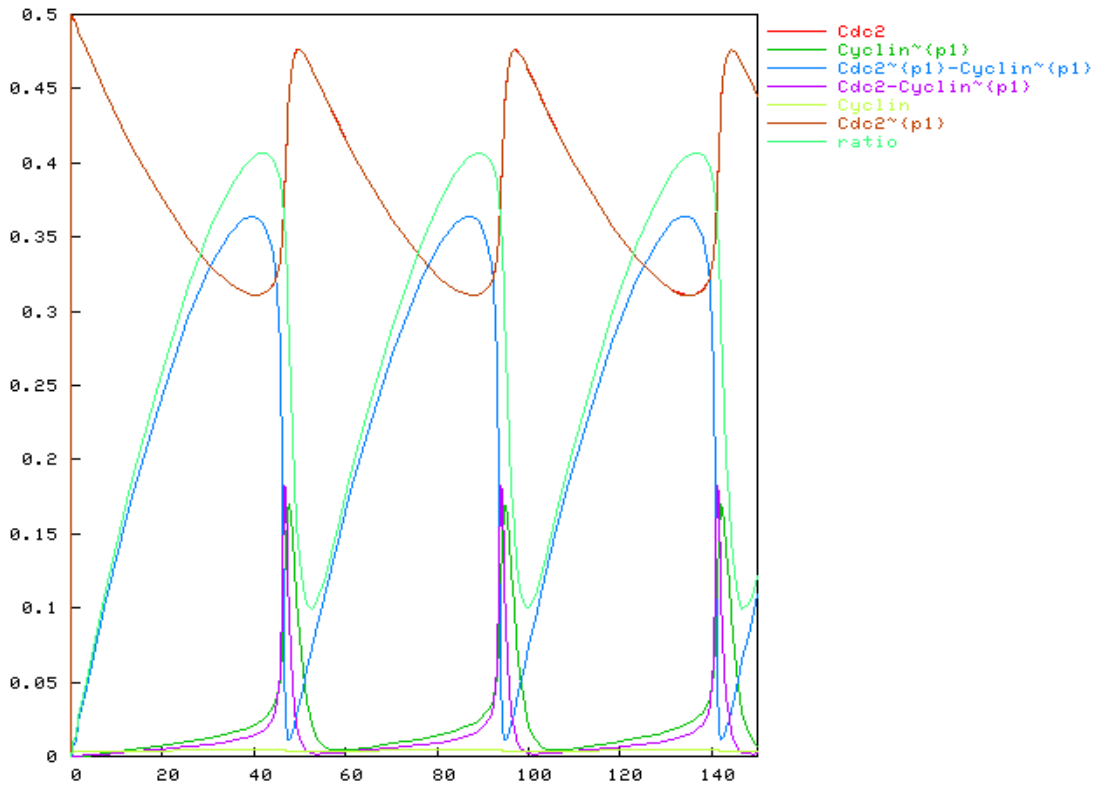
biocham: search_parameter([k3,k4],[(0,200),(0,200)],20,
oscil(Cdc2-Cyclin~{p1},3) & F([Cdc2-Cyclin~{p1}]>0.15), 150).

First values found :

parameter(k3,10).

parameter(k4,120).



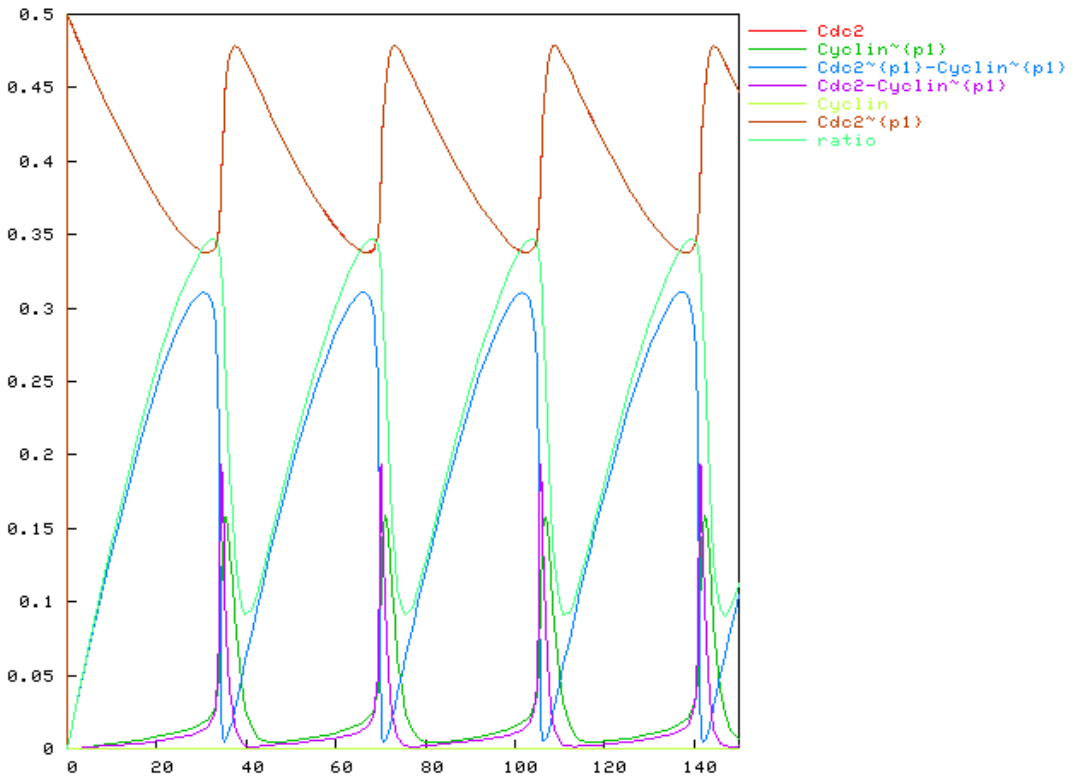
Parameter Search from LTL(R) Properties

biocham: search_parameter([k3,k4],[(0,200),(0,200)],20,
period(Cdc2-Cyclin~{p1},35), 150).

First values found:

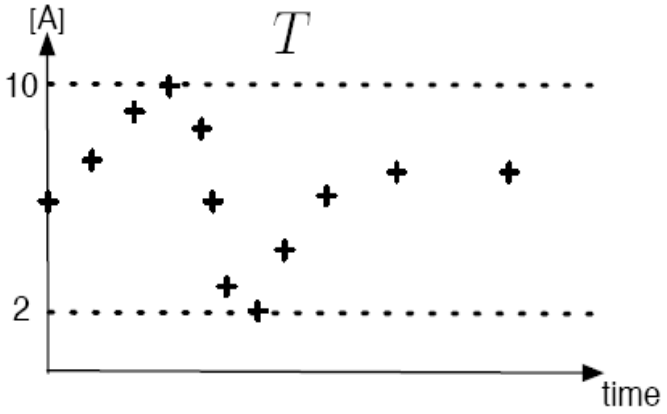
parameter(k3,10).

parameter(k4,280).



But parameter scanning does not scale up beyond 3 parameters...

Model-Checking Generalized to Constraint Solving



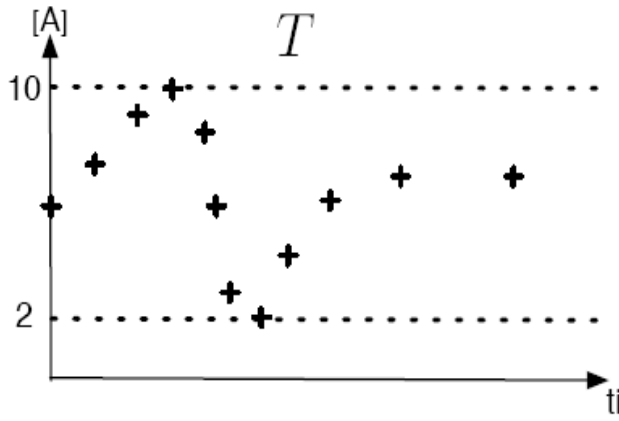
$LTL(\mathbb{R})$

$\Phi = F([A] \geq 7 \wedge F([A] \leq 0))$

Model-checking

the formula is false

Model-Checking Generalized to Constraint Solving



$LTL(\mathbb{R})$

$\Phi = F([A] \geq 7 \wedge F([A] \leq 0))$

Model-checking

the formula is false

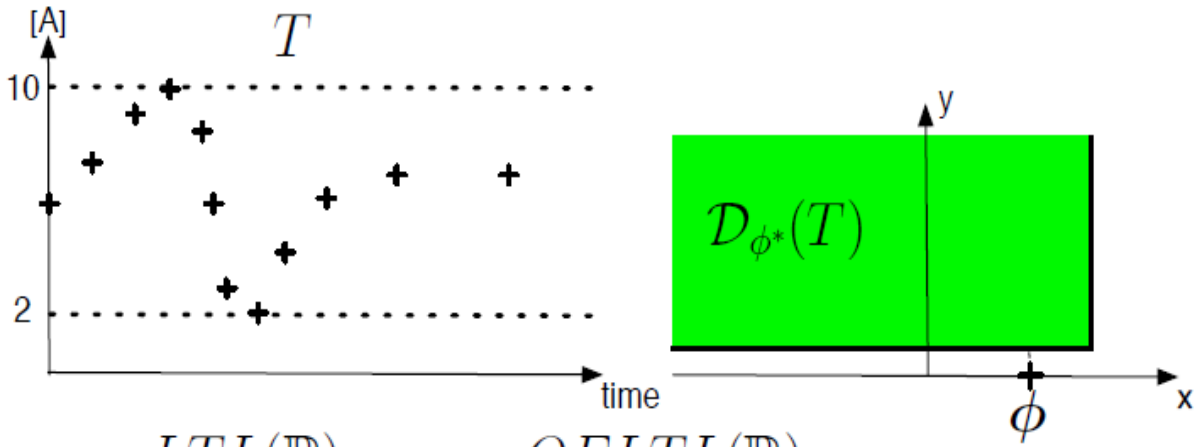
$QFLTL(\mathbb{R})$

$\Phi^* = F([A] \geq x \wedge F([A] \leq y))$

Constraint solving

the formula is true for any $x \leq 10 \wedge y \geq 2$

Model-Checking Generalized to Constraint Solving



$LTL(\mathbb{R})$ | $QFLTL(\mathbb{R})$

Φ=F([A]≥7 ∧F([A]≤0))

Φ*=F([A]≥x ∧F([A]≤y))

Model-checking

the formula is false vd=2 **sd=1/3**

Constraint solving

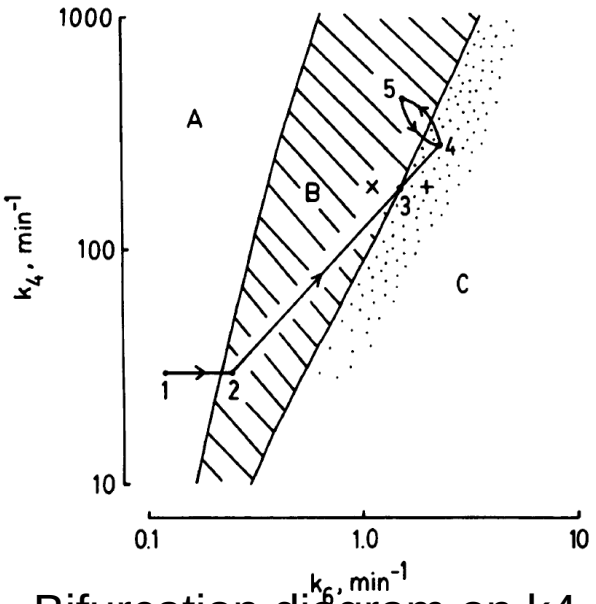
the formula is true for any x≤10 ∧ y≥2

Validity domain $\mathcal{D}_{\phi^*}(T)$ for the **free variables** in ϕ^* [Fages Rizk CMSB'07]

Violation degree $vd(T,\phi) = \text{distance}(val(\phi), D_{\phi^*}(T))$

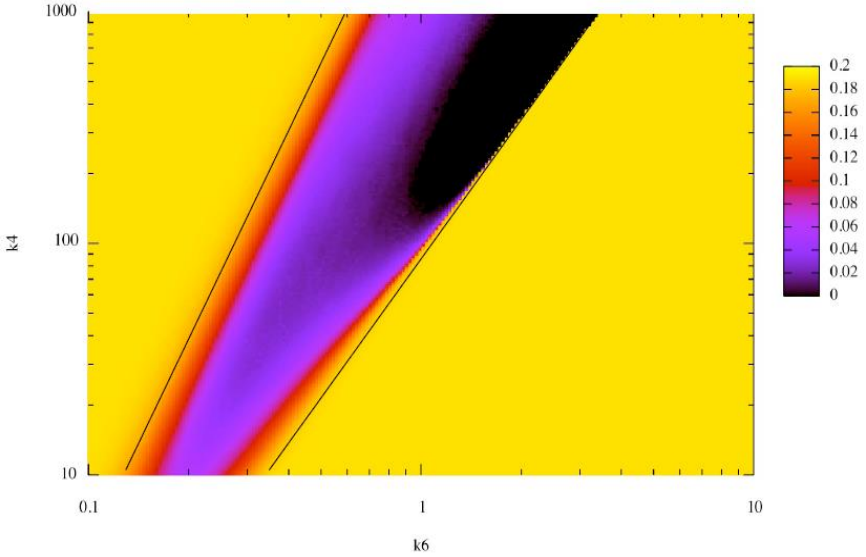
Satisfaction degree $sd(T,\phi) = \frac{1}{1+vd(T,\phi)} \in [0,1]$

LTL(R) Continuous Satisfaction Degree in [0,1]



Bifurcation diagram on k4, k6

[Tyson 91]

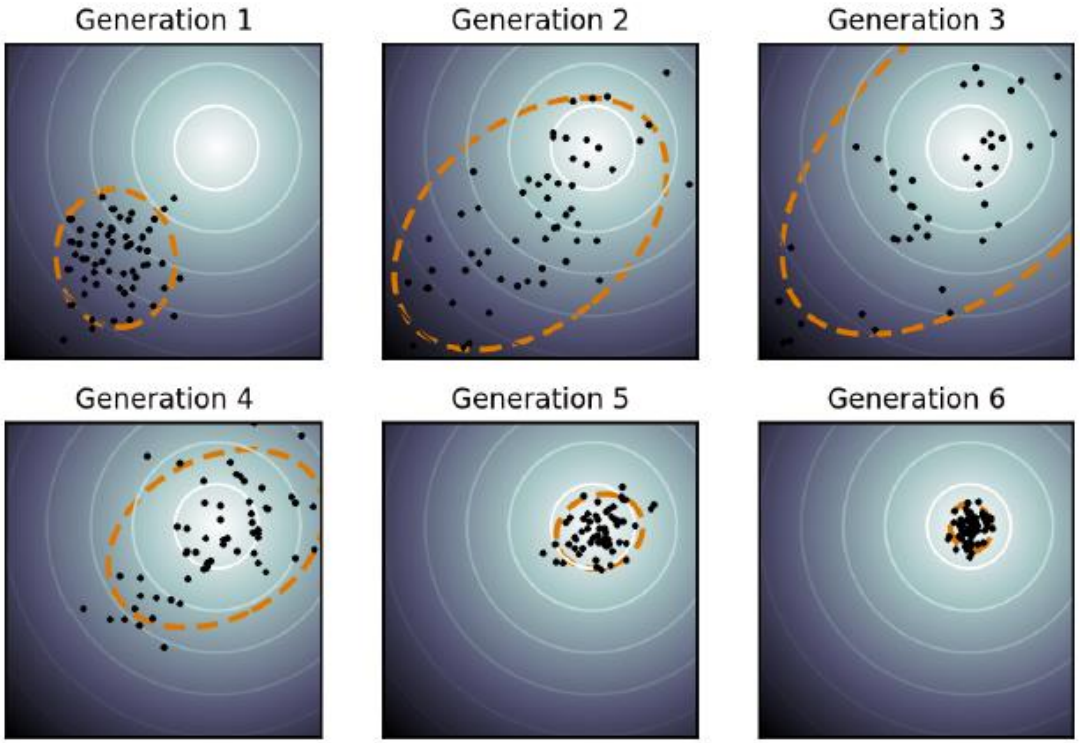


Continuous satisfaction degree in [0,1]
of the LTL(R) formula for oscillation
with amplitude constraint [Rizk Batt FF Soliman CMSB 08]

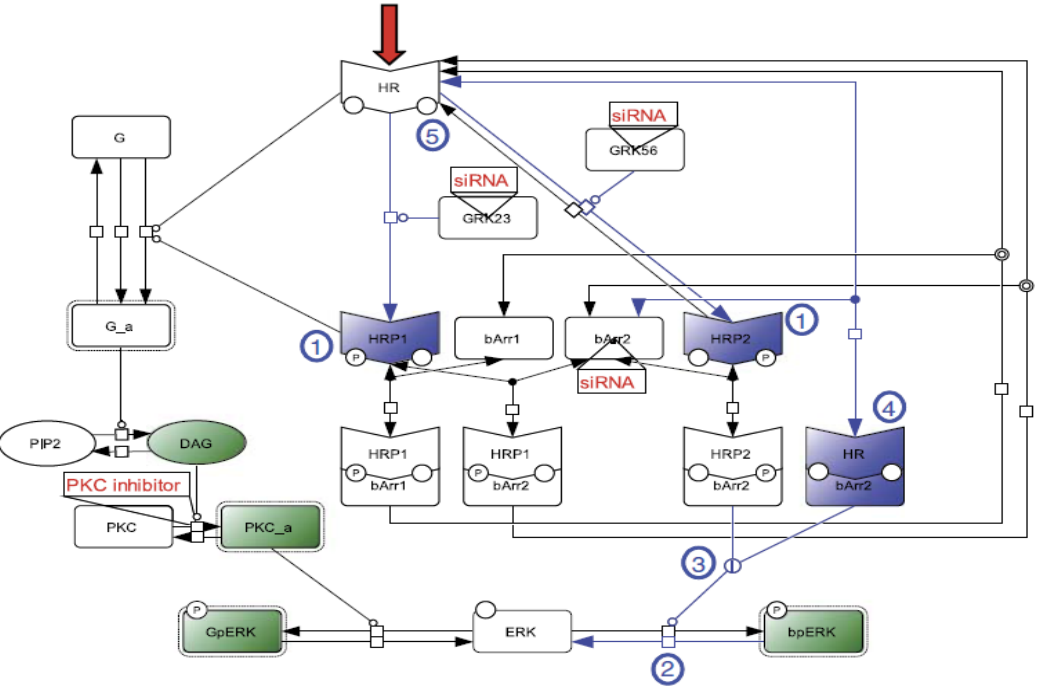
- Parameter search under LTL(R) constraints in high dimension (100 parameters) by continuous optimization (evolutionary algorithm CMA-ES)
- Robustness and sensitivity analyses w.r.t. LTL(R) specification

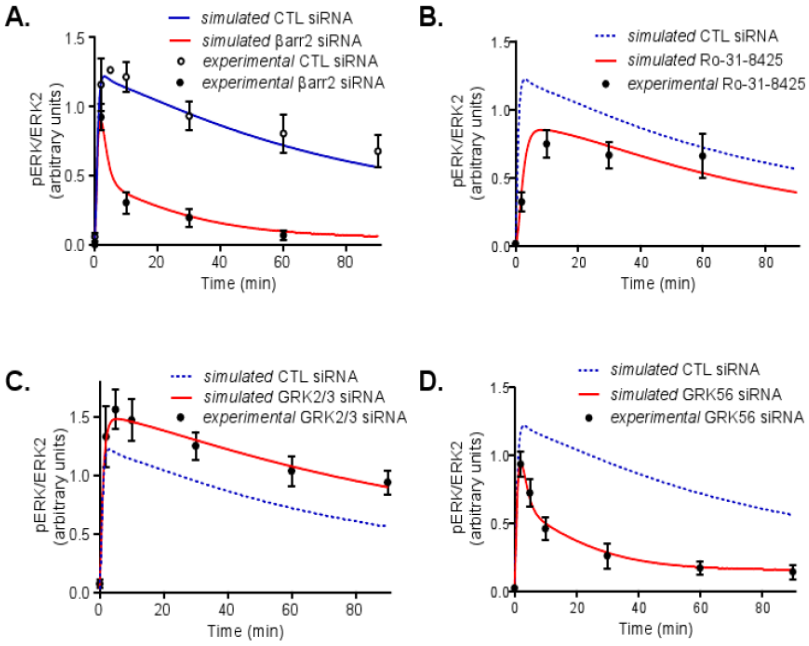
Covariance Matrix Adaptation Evolutionary Strategy

- CMA-ES maximizes a black box fitness function ($sd(\phi)$) in continuous domain ($k_i's$) [Hansen Osermeier 01, Hansen 08]
- CMA-ES uses a probabilistic neighborhood and updates information in covariance matrix at each move



Success Story in GPCR Signaling





Failure to find parameters fitting the data under perturbed conditions in BIOCHAM with CMAES-LTL(R) was the key to revisit the structure of the interactions

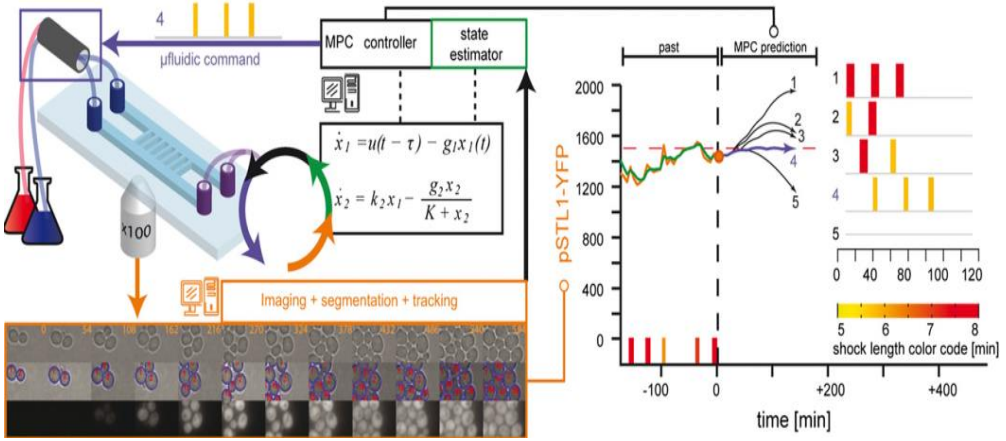
[D. Heitzler, …, FF, R. Lefkowitz, E. Reiter 2012 *Molecular Systems Biology*]

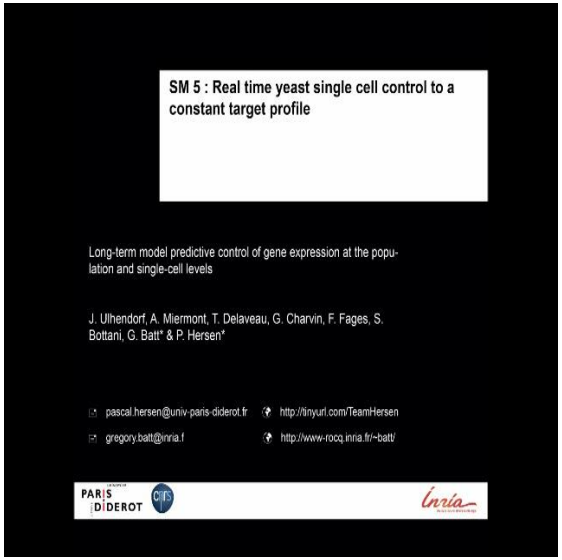
Model-based control of gene expression in yeast

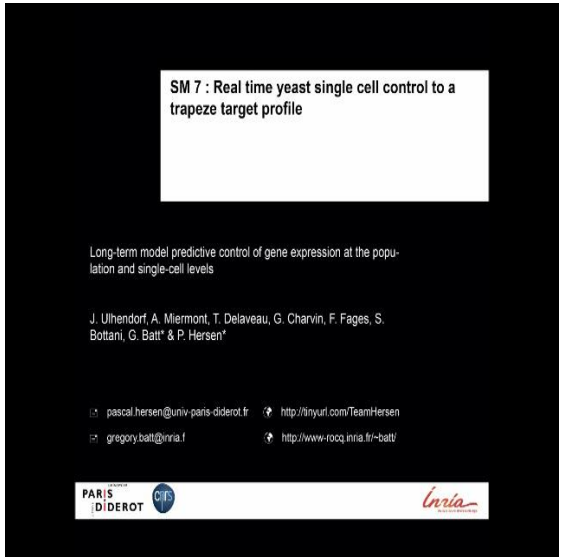
Perception - learning - action loop

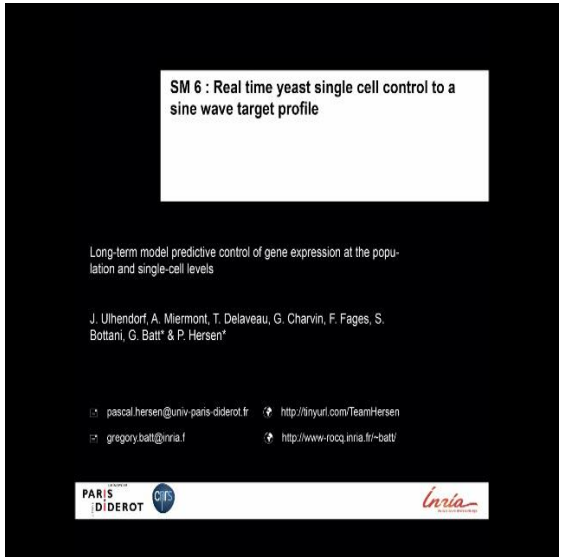
1. Microscope, cell tracking
2. Model fitting (parametrer opt.)
3. Osmotic pressure (parameter opt.)

[Uhlendorf … Batt Hersen PNAS 109(35) 2012]









Wrap-up

- Chemical reaction network theory
  - Sufficient/necessary conditions for dynamical behaviors
  - Hybrid models and hybrid simulations

Crucial for reducing models

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  - Sufficient/necessary conditions for dynamical behaviors
  - Hybrid models and hybrid simulations

Crucial for reducing models

- Static analyzers of reaction programs
  - Structural invariants
  - Model reduction relationships
  - Efficient CLP(FD) and SAT solvers

What can be said from the structure of a reaction network ?

Wrap-up

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What can be said from the structure of a reaction network ?

- Dynamic analyzers of behaviors using temporal logic patterns
  - Parameter search for model building and control

The nerve of war in biological modeling

Wrap-up

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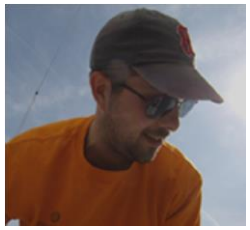
  - Parameter search for model building and control

The nerve of war in biological modeling

- Biochemical programming
  - Synthetic biology: correct or implement new functions in the cell or in non living vesicles
  - Systems biology: elucidate natural circuits as optimized biochemical code

"What I cannot create I do not understand"
R. Feynman

Lifeware team at Inria



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